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Assessment of Radio Protective Effects of *Myrtus communis* L. on Human Lymphocytes Irradiated *in Vitro* by Gamma-rays Using Micronucleus Assay on Binucleated Human Lymphocytes

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Abstract— The study aimed to investigate the potential protective effect of alcoholic extract of *Myrtus communis* L. leaves from the cytogenetic toxicity induced by gamma rays in human peripheral lymphocytes using micronucleus (MN) assay. Irradiation was done at a dose of 2 Gy and the extract was added to cell cultures at two different concentrations 50 and 100 µg/ml an hour before the irradiation of the samples. The results showed that the extract caused a decrease in frequency of MN due to the influence of gamma rays in the concentrations 50 and 100 ug / ml (P = 0.01) compared to the irradiated group. It was observed that concentration 50 µg/ml of the extract caused a greater decrease in the frequency of micronuclei compared to concentration 100 µg/ml, no significant differences were observed in frequency of micronuclei between two groups($p>0.05$).

Keywords— Myrtus communis L., Radioprotective, lymphocytes, gamma radiation, Ionizing radiation, Micronucleus assay, micronuclei.

I. INTRODUCTION

Radiation therapy for cancer, natural and industrial sources of radiation cause great damage to human tissue [1], [2]. Living cells are suffered from damage when are exposed to ionizing radiation causing their death or their conversion into tumor cells, and they may survive because they contain biochemical molecules and mechanisms that repair that damage [3].

Ionizing radiation produces charged ions that carry energy that can penetrate and destroy living tissues, reach cells and destroy the genetic material, which ultimately leads to the inevitable death of cells[4]. Exposure to radiation can cause direct and indirect damage to cells [1], [4],[5],[6]. In direct effect, energy is deposited directly into the target molecule [3] and radiation disrupts chemical bonds in the cell [6].

Free radicals are formed as a result of the dissolution of water molecules in the living cell by the action of radiation. These Free radicals cause damage and harm to cells and this is what we call the indirect effect of radiation [4],[6],[7]. Reactive oxygen species caused by radiation attack various cellular molecules [7]. Damage to critical molecules such as DNA can occur and may damage DNA, mutations, and cancer [1],[2],[5] when Ros interacts with biomolecules, it can produce secondary free radicals which lead to toxic cell events [4].

The rays cause the DNA chains to be fragmented, resulting in a set of chromosomal aberrations in the genetic material [8] and these Breaks are the cause of Chromosomal aberrations [9], such as micronuclei and other chromosomal aberrations [9], [10]. Myrtle belongs to the Myrtaceae family and is considered one of the plant species of high importance due to its many uses in different life fields. It is used medicinally to treat many diseases and is also used as a spice to improve the taste of foods.

The plant appears in the form of small shrubs with a height of 1.8-2.4 meters, and it is one of the plant species widely spread in the areas surrounding the Mediterranean Sea [11].

Many important compounds and substances have been found in the oils extracted from plants [12],[13],[14],[15]. Different compounds provide the plant with many medicinal and therapeutic benefits for many pathological disorders, and the most important components found in the studied plant are phenolic compounds, which include a group of compounds such as phenolic acids, flavonoids, and tannins [16], [17], [18], [19], [20], [21] in addition to monoterpenes [19] and other materials such as catechin, quercetin, and quercetin, which are compounds of the plant family to which it belongs To the plant studied in this paper [11], [22], [23]. It was found that the extract have multiple drug efficacy and the plant has been used to treat many disorders including inflammation [24] such as

urinary tract infection, bronchi and sinusitis, cough, gastrointestinal problems, hemorrhoids, diarrhea, cramping, rheumatism, neurological problems. Such as epilepsy, analgesic, hypoglycemic [25], [26] antihypertensive [27] antibacterial [28] and microbes [25], [28] and parasites [25] anti-anxiety, acne, herbicide, and antifungal [29].

Candidiasis is [30] anti-malaria [31] Reducing the risk of atherosclerosis [32] improving immune performance [33] anti-toxicological effects [25] in addition to the anti-cancer properties against many different cancer cell lines such as leukemia [34] bladder cancer [35] breast cancer [36] prostate cancer [37]. In addition to the antioxidant properties [38], [39], [40], interest in natural products began to reduce the negative impact of radiation [7] as the components of their medicinal plants Important in Reducing the negative and dangerous effects resulting from radiation, and this effect is due to the high content of compounds that have antioxidant properties that inhibit free radicals and nullify their effect of causing the so-called oxidative stress that causes extensive damage to living cells, which protects the cell from damage, destruction, and death [4].

Radioprotective materials neutralize free radicals, thereby reducing the occurrence of primary radiological effects following radiation exposure [7]. The antioxidant activity is attributed to the presence of phenol, flavonoids, and tannins in the various parts of the *Myrtus communis* L.[38] , The antioxidant properties are due to two factors, first the number of hydroxyl groups and secondly the binding sites of these groups in the molecules that make up the phenolic compounds [11].

Polyphenol inhibits free radical damage to fats and proteins [41], prevents harmful oxidation, and protects against toxic effects Free radicals reduce their capacity and transform them into more stable products by giving electrons [27] and hydrogen to interactive free radicals [42].

II. RELATED WORK

Most articles have examined the antioxidant effect of exponent extracts against the induction of various chemical compounds for oxidative stress and have shown that the phenolic part of the plant has had an antioxidant effect by activating antioxidant enzymes, inhibiting the activity of pro-oxidant enzymes, activating DNA restoration enzymes and reducing lipid peroxide [4],[32],[43] Accordingly, the researchers concluded that it can protect cells from oxidative stress.

We, therefore, believe that the extracts may have a protective effect from ionizing radiation that stimulates oxidative stress and DNA damage. Accordingly, the importance of this research stems from the fact that it will investigate the effect of alcoholic extracts of *Myrtus communis* L. leaves on radiation sensitivity and determine the mechanisms of radiation protection for the treatment of leaf extracts on the circuit and mitigate cellular genetic damage from This study confirms that this study has a scientific and applied medical significance that is demonstrated by the possibility of using the plant as a radiation shield for workers in the field of ionizing radiation and the side effects of radiotherapy and for the mitigation of oxidative stress induced by cancer.

In this research, we studied the radiation protective effect of *Myrtus communis* L. leaves extract by adding an alcoholic extract to myrtle leaves in concentrations of 50 µg/ml and 100 µg/ml to human lymphocyte culture an hour before exposing cells to ionizing radiation.

III. METHODOLOGY

Plant and extract preparation:
Leaves of *Myrtus communis* L. were collected from natural habitats, in Damascus countryside, in the Syrian Arab Republic, during October-November 2018. The plants were identified by the plant taxonomist professor M. Oddat, at the Department of radiation protection, the Syrian Atomic Energy Commission.

500 ml of ethyl alcohol was added at a concentration of 70% to 50 g of dried *Myrtus communis* L. leaves in the shade at the laboratory temperature, then extracted twice in alcohol each time for 24 hours on the same leaves for 48 hours, then the resulting extract was taken and the alcohol was vaporized from it At 41 ° C for 3 days, the final extract was finally obtained and then refrigerated at -20 ° C until use.

irradiation:
Gamma irradiation was performed with a gamma-cobalt-60 (60CO) source at the standard laboratory of the Radiation Protection Department of the Syrian Atomic Energy Commission, in a water bath set at 37 ° C, at a dose of 2 Gy.

Sampling:
We collected 15 ml of blood from a forearm vein from five non-pathogenic and non-smoking donors(2 male and 3 female) using sterile, vacuum tubes containing lithium heparin, which acts as an anticoagulant.
Then each sample was divided into two parts. The first part was irradiated with a dose of Gy, while the second part was left without irradiation, then cultivation was performed.

Lymphocyte culture:
Lymphocytes were cultured according to the Sea bright and Frank with some modifications, and the method consisted of a set of standardized steps provided by the International Atomic Energy Agency, and each of [44] and [45] mentioned the method.

Cell Treatment:
Each blood sample was divided into six equal parts. The concentrated of the extract was added to the PBMCs with a

single treatment as the following: The first was left as a control, the second was irradiated with 2 Gy. of Gamma-ray, the third was treated with 50 µg/ml of the extract, The fourth part was treated with 100µg/ml of the extract, The fifth part was treated with 50 µg/ml of the extract 1 h. before irradiation and The sixth part was treated with 100µg/ml of the extract 1 h. before irradiation. Then, cell culture was performed.

Statistical analysis:

In this work, the results were shown as mean ± standard deviation (mean ± SD), and we analyzed the results statistically using Excel 2003) using T-Test (Two-Sample Assuming Equal Variances). This method is used in general to estimate the differences between two statistic groups in The case of small samples (n <30), to know the differences between the two societies, are they significant differences with 95% confidence level? It adopted the value of P-Value as a statistically significant value when it was ($p \leq 0.05$), or not significant when it was ($p \geq 0.05$).

IV. RESULTS AND DISCUSSION

When applying the steps for culturing lymphocytes mentioned in the section on culturing lymphocytes, bi-nucleus lymphocyte cells were obtained in clear shapes and appropriate numbers for microscopic study. Figures 1 and 2 show some microscopic images of bi-nucleuslymphocytes and bi-nucleus lymphocytes containing micronuclei obtained in this study, were 500 bi-nucleus lymphocytes were studied for each concentration of the alcoholic extract of the leaves of *Myrtus communis* L. plant and each of the five donors.

The number of micronuclei in bi-nucleus lymphocytes obtained from culture of peripheral blood lymphocytes were studied for five healthy, non-exposed donors. Figures 3, 4, 5, and 6 appear effect of different concentrations of alcoholic extract of *Myrtus communis* L. leaves and irradiation on the frequency of micronuclei in peripheral blood lymphocytes for all donors in several experimental points [negative control, positive control includes the extract only with a concentration of 50 µg/ml, positive control includes the extract only with a concentration of 100 µg/ml, Radiant control, Radiated an hour after the bosom with the extract at a concentration of 50 µg/ml, irradiated an hour after the bosom with the extract at a concentration of 100 µg/ml]**.**

Figure (3) shows a graphical representation of frequency of micronuclei in the control sample and the samples treated with the extract only. The percentage of the number of micronuclei of the control group was (0.035 ± 0.024). The treatment with alcoholic extract of *Myrtus communis* L. leaves with a concentration of 50µg/ml reduced the frequency of the micronuclei by comparison. With the control group (0.029 ± 0.013), treatment with alcohol extract of *Myrtus communis* L. leaves with a concentration of 100 µg/ml also resulted in a decrease in frequency of micronuclei compared to control group (0.030 ± 0.017), but this decrease using the previous two concentrations was not statistically significant compared with the Control group (P = 0.3 ,P = 0.3 in series).

The radiation alone, as shown in Figure 4, resulted in a significant increase in frequency of micronuclei (0.171 ± 0.057) compared to the control group (0.030 ± 0.017) and the increase was statistically significant (P = 0.00).

Figure 5 shows that adding alcoholic extract to leaves of *Myrtus communis* L. plant to the cells at a concentration of 50 µg/ml an hour before irradiation resulted in a significant decrease in frequency of micronuclei (0.099 ± 0.031) compared to the radioactive group(0.171 ± 0.057) and the decrease was statistically significant (P = 0.01).

Figure (6) shows that adding alcoholic extract of *Myrtus communis* L. leaves to the cells at a concentration of 100 micrograms 1 hour before radiation has resulted in a significant decrease in frequency of micronuclei (0.112 ± 0.054) compared to the radioactive group (0.171 ± 0.057). This decrease was statistically significant (P = 0.01).

As is evident from Figures 5 and 6, the addition of an alcoholic extract to leaves of *Myrtus communis* L. plant to the lymphocytes at concentrations 50 and 100 µg/ml an hour before irradiation resulted in a significant and statistically significant decrease (P = 0.01) in frequency of micronuclei induced gamma rays compared to the radiating group. It is also noted that adding the extract to cellular cultures at a concentration of 50 µg/ml resulted in a greater decrease in frequency of micronuclei compared to the concentration of 100 µg/ml, although these differences were not significant (P = 0.29).

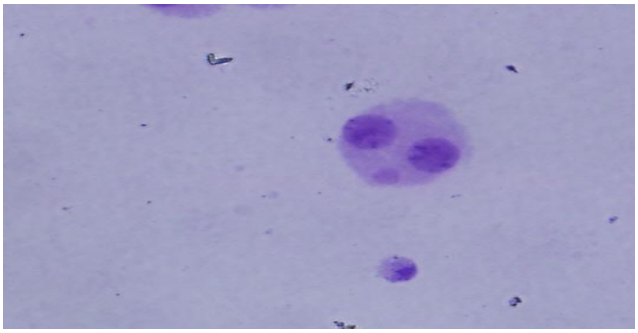

Figure 1: A micrograph of binucleated cells with one micronuclei.

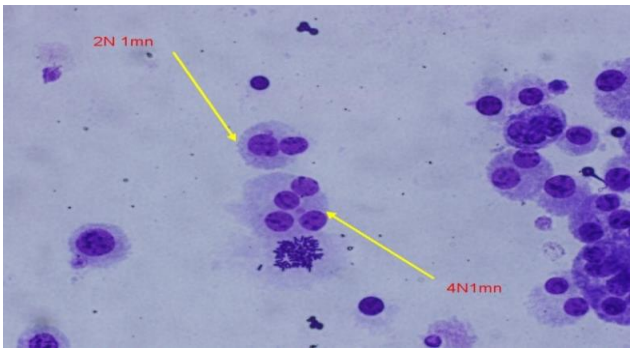

Figure 2: A micrograph of a normal binucleatedcell (2N), binucleated cell with a micronucleus (2N 1mn) and a Tetra nucleate cell with a micronucleus (4N 1mn).

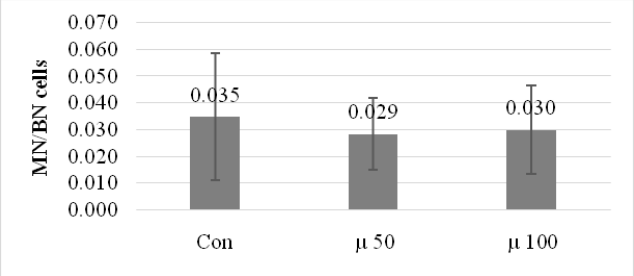

Figure 3. The frequency of Micronucleus in cultured human lymphocytes treated for 1 h. with 50 µg/ml or 100 µg/ml of alcoholic extract of Myrtus communis L.

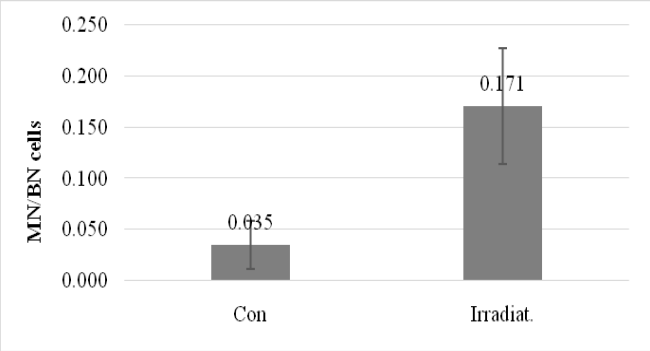

Figure 4: Effect of irradiation with 2Gyofgamma rays on frequency of micronuclei in peripheral blood lymphocytes.

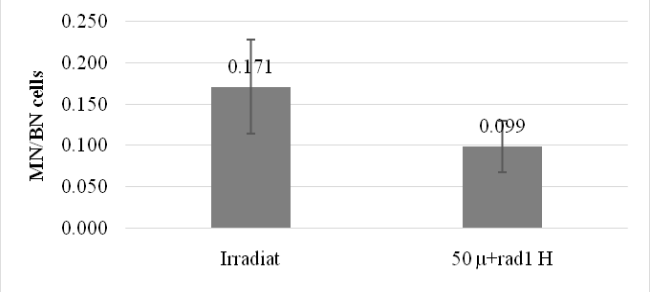

Figure 5. The frequency of Micronucleus in cultured human lymphocytes treated with 50 µg/ml of alcoholic extract of Myrtus communis 1 h. before irradiation with 2 Gy of Gamma-ray.

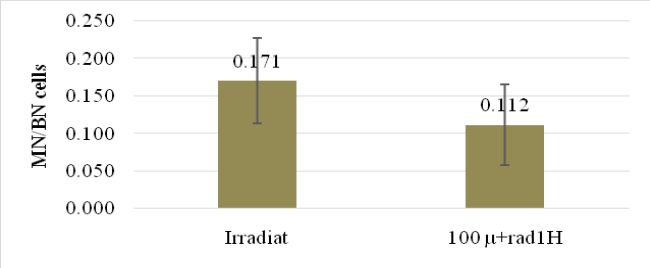

Figure 6. The frequency of Micronucleus in cultured human lymphocytes treated with 100 µg/ml of alcoholic extract of Myrtus communis 1 h. before irradiation with 2 Gy of Gamma-ray.

Discussion

Ionizing radiation causes oxidative stress in cells by enhancing the formation of Ros, and interest in natural antioxidants has begun as rates of radiation effect and despite this, most of the results obtained from various studies on antioxidant compounds were inconclusive [7]..In this study, we have adopted the method of Micronucleus Assay, which is one of the most successful and reliable, assays in the genetic toxicity test and has been widely used to investigate various possible effects of Radioprotective materials [2].

We assayed micronuclei induced with a 2 Gy dose of gamma rays in the peripheral blood of five healthy, non-exposed Ionizers. We examined the effect of adding two concentrations of alcoholic extract to leaves of *Myrtus communis* L. plant [50and 100µg/ml], an hour before irradiation, on modifying gamma-ray induction of micronucleiin peripheral blood lymphocytes in the glass. The results showed, in general, that extract of plant significantly reduced the genetic cellular damage induced by gamma rays in human lymphocytes.

Where it was found that adding the extract (whether 50 or 100 µg/ml) to cell cultures causes a decrease (P = 0.3) The number of micronuclei if we compare it with an untreated control with gamma rays(P = 0.3), which indicates That this extract is low toxic and does not cause DNA damage, This result is similar to the results obtained by (1)in their research on the effect of green tea on modifying the effect of gamma rays on human lymphocytes.

Irradiation induced a significant increase (P = 0.00) in frequency of micronuclei in peripheral blood lymphocytes approximately 5 times frequency recorded in the non-irradiated control group.

We can explain this increase in the formation of chromosomal aberrations that results from the fragmentation of the genetic material by the effect of ionizing rays and this result was confirmed by each of [9] and [8]in their studies that dealt with the effect of rays on human lymphocytes.

The incubation of lymphocytes with two concentrations of 50 and 100µg/ml with alcoholic extract of *Myrtus communis* L. leaves an hour before irradiation Decreasing the percentage of micronuclei by up to half the number formed in cells by radiation (P = 0.01).

We believe that the Radioprotective properties of *Myrtus communis* L. plant are due to the high antioxidant ability. With its high content of polyphenols, it is able to neutralize the interactive free radicals caused by radiation by giving them electrons and hydrogen [27], [41],[42] and found that the phenolic part of the plant had an antioxidant effect by activating antioxidant enzymes, inhibiting the activity of pro-oxidant enzymes, activating DNA restoration enzymes and reducing lipid peroxide, [4],[32],[43].

The results of this study are consistent with studies carried out by [1],[8], [5], [46],[47],[48], on the effect of the phenolic content of rosemary, green tea, Rutin and Quercetin, and Apigenin and Flavan3OLs in grape seeds on modifying effect of gamma rays on human lymphocytes as their studies demonstrated the protective and anti-

oxidant potentials and the ability to neutralize the free radicals of these. Plants and compounds.

The results obtained are also consistent with results of [49] in their study on extracts of leaves of Syzygium cumini, which belongs to the Myrtaceae family, which showed that Syzygium cumini plant reduces frequency of micronuclei and damage of DNA caused by radiation in human lymphocytes, And with results of [4] in their study on effect of many plant extracts rich in polyphenols.It was observed that the extract at the concentration 50 µg/ml caused a greater decrease in frequency of the micronuclei of the concentration 100 µg/ml as is apparent from the low genetic toxicity in this concentration and there was no benefit By increasing the concentration, therefore, the optimal concentration of alcoholic extract of *Myrtus communis* L. leaves in this study was 50 µg/ml.

V. CONCLUSION AND FUTURE SCOPE

We note from the foregoing the ability of the *Myrtus communis* L. to reduce the effect of gamma rays, as the extract used in two different concentrations showed a high ability to reduce the frequency of micronuclei and the toxic effects induced by radiation in the lymphocytes of human donors when adding the studied extract before exposing the cellular samples to radiation for an hour. The absence of negative effects and side effects of the extract on the vitality of cells was found, and the study provides strong evidence about the effectiveness. The serious results that we are getting there must be more in-depth studies on the plant Before the plant was approved as a radiation shield.

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